UNIVERSIDADE CATÓLICA PORTUGUESA | INSTITUTO DE CIÊNCIAS DA SAÚDE

ACCURACY OF DIAGNOSTIC TESTS FOR LEGIONNAIRES' DISEASE

Dissertação apresentada à Universidade Católica Portuguesa para obtenção do grau de mestre em

INFECÇÃO EM CUIDADOS DE SAÚDE

Por

Elisabete Cristovam Santos

(Lisboa, 2013)

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Sob a orientação de

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e

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ACCURACY OF DIAGNOSTIC TESTS FOR LEGIONNAIRES´ DISEASE

ABSTRACT

Background

Legionellosis are infections caused by *Legionella* spp. The diagnosis of high-risk patients should rely on microbiological tests which allow the establishment of this infection etiology. Cases have to be confirmed through the available diagnostic methods which have different performances, sensitivity, specificity, error causes, limitations, and needs of careful interpretation.

Objectives

Assess the accuracy of urinary antigen detection, direct fluorescent antibody (DFA) staining, serological testing, Protein Chain Reaction (PCR) versus culture (reference standard), in patients suspected to be infected with *Leggionella* or patients with laboratory confirmed Legionnaire Disease (LD).

Search Methods

We searched the Cochrane Central Register of Controlled Trials (CENTRAL) in The Cochrane Library and MEDLINE (PubMed). We also handsearched the reference lists of the included studies.

Selection criteria

Observational studies were included, comparing the index tests with culture in patients suspected to be infected with *Legionella* or patients with laboratory confirmed LD.

Data collection and analysis

Two authors independently assessed the trials and extracted data. Data was analysed using statistical software Review Manager 5.1.

Main results

Five studies met the inclusion criteria. All studies evaluated PCR and DFA tests to detect *Legionella* in clinical specimens, comparing it with culture (reference standard) and were included in meta-analysis. PCR sensitivity and specificity ranged from 56% to 100% and from 89% to 100%, respectively. The pooled sensitivity was 74% (95% IC 67%-80%), and the specificity 97% (95%IC 96%-80%). DFA sensitivity varied from 33% to 44% and the specificity from 100% the pooled sensitivity was 40% (95% IC 21%-61%) and the specificity 100% (95% IC 81%-100%).

Author´s conclusions

This review demonstrates that PCR have a high sensitivity and specificity for early diagnosis of LD. However standardization is required for biological samples. Although this, culture is always required for epidemiological studies, strains molecular typing and antibiotic sensibility evaluations if needed.

BACKGROUND

Description of the Condition

Legionellosis are infections caused by *Legionella* spp. Classically presents two distinct forms: Legionnaires' disease (LD), a serious and potentially life-threatening illness which includes pneumonia, and Pontiac Fever, a milder febrile flu-like illness without pneumonia. (Zaragoulidis 2011). Most of the LD patients present fever, non-productive cough, headache, myalgias and dyspnea. Clinical syndroms may include diarrhea, nausea, vomiting, liver and kidney dysfunction, thrombocytopenia, hypophosphatemia and neurological disorders. The diagnosis of high-risk patients should rely on microbiological tests which allow the establishment of etiology of this infection (Zaragoulidis 2011; Fields 2002). These tests should be specifically requested, as they are not routinely performed in laboratory (Murdock 2010). All cases have to be confirmed through the available methodology that includes, classical culture (gold standard), direct fluorescent antibody assay (DFA), urinary antigen detection, serological assay and nucleic acid amplification (Silva 1996). Those techniques have different performances, sensitivity, specificity, error causes, limitations, and needs a careful interpretation (Fields 2002).
A confirmed case requires one of the following criteria: isolation of *Legionella* spp. in a clinical sample, antibody titer increase (4x) being the second titer not less than 128 for *Legionella pneumophila* serogroup 1 or urinary positive antigen for *Legionella pneumophila* serogroup 1 (Silva 1996).

Epidemiology and Pathogenesis

Legionella spp are pleomorphic Gram negative motile rods. They are intracellular parasites, strict aerobic, nutritionally demanding, cysteine require for their growth. Some species are able to survive between 20 ° C and 45 ° C. *Legionella* species are ubiquitous in both natural and artificial aquatic environments. (Zaragoulidis 2011). However these bacteria infrequently cause disease. There must be a combination of factors to further LD infection. They include: the proper environmental conditions allowing virulent strains survival; a way of bacteria dissemination such as aerosolization; and inhalation of an infectious dose by a susceptible host. The main mode of transmission of these infections is the airway, inhalation of aerosols containing *Legionella,* or micro aspiration of contaminated water. There is no evidence of direct transmission from person-to-person (WHO 2011). Host risk factors associated with this infection are diverse, such as male gender, older than 50 years, smoking, underlying predisposing conditions as chronic obstructive pulmonary disease, immunosupression associated with cancer or solid organ transplantation and therapy with high doses of corticosteroids (Mandell 2010).

Impact

Severe infections are frequently associated with immunocompromised patients. According to European Legionnaires´ Disease Surveillance Network (2013), although under-diagnosed and under-reported in all countries, in European countries, the overall notification rate was 11.5 per million inhab in 2012, whereas 69% are for cases of community-acquired disease, 12% travel -associated (within and outside the country), 8% healthcare associated and 3% of others settings. World Health Organization data suggest a high mortality rate 40-80% associated with untreated immunocompromised patients (ELDSNet 2013). These data can be reduced to 5 to 30% through appropriate case management and depending on the severity of the clinical signs and symptoms (WHO 2011).

Index test (s)

Culture

Culture remains the reference method, Gold Standard, for *Legionella* spp, diagnosis with a specificity of 100%. Estimated sensitivity culture of clinical samples range from <10% to 80%, probably due previously empirical therapies that may inhibit growth in vitro. Culture diagnosis requires special media, adequate processing of specimens and technical expertise. The standard medium used is buffered charcoal yeast extract (BCYE) agar which provides iron and L-cysteine, with or without antibiotics, essentials for the growth of *Legionella*. The appearance of *Legionella* on the surface of BCYE is characteristic: colonies resembling cut glass, when observed with magnifying glass (40x). Two days are usually required for recognition of colonies, but most *Legionella* colonies can only be detected after 3-5 days. (Mandell 2010; Silva 1996).

Direct fluorescent antibody (DFA) staining

DFA is a diagnostic test used for detection of *Legionella pneumophila* antigen directly in respiratory specimens and tissue samples. This technique has the advantage of providing a result within 2-3h which allows preliminary information useful in guiding treatment. Method with high specificity estimated at 94% although can be less specific with

inexperienced laboratory personnel, due the possibility of cross-reactions with other bacteria, including *Pseudomonas spp.* Values of sensitivity have a range of 25 to 70% once is technical demanding and depending on sputum sample. For this all reasons DFA is only considered a probable diagnosis of *Legionella* infections according to European Working Group for Legionella Infections (EWGLI) (Fields 2002; Murdoch 2003; Pedro-Botet 2011).

Urinary antigen detection

Urinary antigen detection used worldwide allows a confirmed diagnosis of *Legionella pneumophila* serogroup 1. Up to date, several commercially available tests have been developed for detection of *Legionella pneumophila* serogroup 1 antigen in urine, such as Enzyme Immunoassay (EIA) and Immunochromatographic (IC) assays. The EIA method is not used in routine because is laborious and require specific equipment, on other hand IC is a rapid technique, requires no laboratory equipment and the results are obtained within 15 minutes (Murdoch 2003; WHO 2011). This test has specificity (100%) and sensitivity (70-90%) which can be enhanced after a prolonged incubation time until 60 minutes. (Pedro-Botet 2011)

Serological testing

Detection of antibodies of *Legionella* in serum by indirect immunofluorescence (IFA) has a sensitivity range between 78 to 91% and specificity of 90% (Diagnosis of Legionella infection). There are some limitations, such as the need to obtain serum samples taken at intervals of at least 3-4 weeks to check seroconversion – a 4-fold or greater increase in antibody titer. Other disadvantages are the inability to accurately detect all *Legionella* species and serogroups, registration of cross-reactions and variations in the kinetics of the antibody, which is poorly known (Fields 2002; WHO 2011).

Nucleic Acids amplification

PCR has been successfully used to detect *Legionella* DNA in clinical and environmental samples and are promising for a rapid diagnosis of legionellosis. There are several techniques available using rRNA (ribosomal RNA): rRNA 5S, 16S rRNA gene mip (macrophage infectivity potentiator) among others, which makes a non standardized method due to different degrees of sensitivity and specificity. Therefore, any result should be interpreted as a probable diagnosis (Project). The technique of real time PCR (RT-PCR) combined with hybridization probe enables the specific amplification of *Legionella* DNA, providing results in a short time and confirming the reduction cases of cross-contamination (Murdock 2010).

Why is important to do this review

It is extremely important to obtain a rapid and effective diagnosis of LD cases to provide timely and appropriate therapy, to decrease the morbidity and mortality rates and to reduce costs associated with this disease. Culture is the reference standard for LD, although essential in clinical and epidemiological research, is time consuming and has a low sensitivity not allowing a rapid diagnosis in a severe case of illness. We, therefore, conducted a systematic review of the literature to estimate the accuracy of several diagnostic tests compared with culture, for the diagnosis of LD.

OBJECTIVES

To assess the accuracy of urinary antigen detection, direct fluorescent antibody (DFA) staining, serological testing, Protein Chain Reaction (PCR) versus culture (reference standard), in patients suspected of suffering from LD or patients with laboratory confirmed LD.

METHODS

Criteria for selecting studies for this review

Types of studies

We aimed to include studies published in English. Studies were considered eligible if diagnostic methods were evaluated in the same patient (direct comparison) against the reference standard- culture; absolute numbers of true-positive, false-negative, true-negative, and false-positive observations were available or could be derived from the reported data.

Participants

Patients suspected of infection with *Legionella* or patients with laboratory confirmed LD.

Index tests

- Urinary antigen detection;
- Direct fluorescent antibody (DFA) staining;
- Serological testing;
- Protein Chain Reaction (PCR)

Comparator tests

Any of the listed index tests were compared with the reference standard for LD.

Target conditions

Legionnaires´disease.

Reference standard

Culture.

Search methods for identification of studies

Electronic searches

We identified eligible studies by searching the following databases:

- The Cochrane Central Register of Controlled Trials (CENTRAL) in The Cochrane Library using the search strategy in Appendix 1;
- MEDLINE (PubMed) using the search strategy in Appendix 2.

Both searches were limited to studies published in the English language. We also restricted our search to published studies. We screened titles, keywords and abstracts of retrieved articles from the electronic searches, and obtained full copies of reports of potentially relevant trials for further assessment.

Searching other resources

We handsearched the reference lists of all primary studies identified by the initial search.

Data collection and analysis

Selection of studies

Two review authors (EC, DA) independently assessed the studies identified by the search strategy, to identify potentially relevant trials for the review according to the criteria outlined above. We resolved disagreements about inclusions by discussion.

Data extraction and management

A standardised data extraction form was used to abstract study design features and results data from each publication. For each study data were extracted independently by two authors (EC, DA). We extracted: year of publication, aim of study, study design, clinical setting, population, outcomes and the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) methodological items (Reitsma, 2009). We also recorded the numbers of true positives, true negatives, false positives and false negatives

Assessment of methodological quality

Methodological quality of included primary studies was assessed by two authors using a modified QUADAS tool (Whiting 2003) that included 11 of the 14 mandatory items, representative spectrum, acceptable reference standard, acceptable delay between tests, partial verification avoided, differential verification avoided, incorporation avoided, reference standard results blinded, index test results blinded, relevant clinical information, uninterpretable results explained, withdrawals explained. The operational definitions of the QUADAS items are presented in Appendix 3.
Extracted data were used to estimate sensitivity and specificity and to investigate the diagnostic performance of each index test.

Statistical analysis and data synthesis

We extracted or derived data of diagnostic performance presented in each primary study for each index test. Data was analysed using statistical software, Review Manager 5.1. We constructed 2 X 2 tables of true positive cases, false positive cases, false negative cases, and true negative cases. We considered patients with culture-positive results as true positives and patients with culture-negative results as true negatives when analysing the performance of each index test. We calculated sensitivity and specificity with 95% confidence intervals (CI) in each study and create forest plots and hierarchical summary receiver operating characteristic (HSROC) of sensitivity and specificity for each index test to investigate the diagnostic performance of each index test and heterogeneity in the diagnostic performance of each index test. Where there were studies of similar comparisons reporting the same outcome measures, a meta-analysis was undertaken.

Investigations of heterogeneity

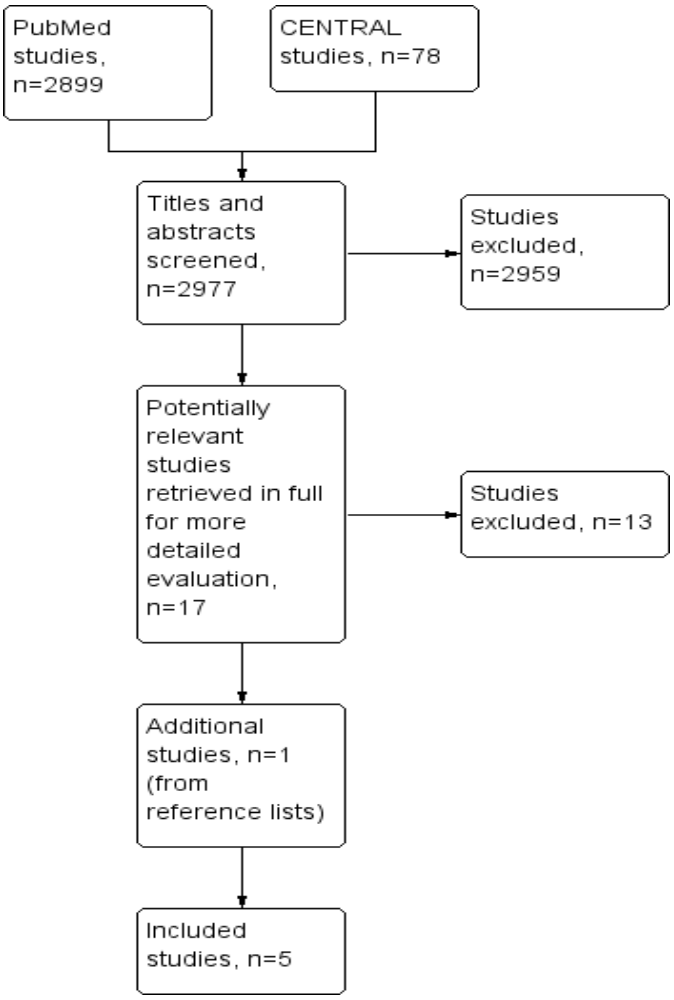
Factors that could influence diagnostic accuracy other than true test performance included those relating to methodological quality and study design, characteristics of the underlying population, and characteristics of the index and reference test. We detailed and compared patient inclusion criteria for each included study. Factors such as differences in study population characteristics and test application (criteria for positive test) were used to explore any heterogeneity discovered in the analysis for the test, and to assess the impact of heterogeneity on the relative accuracy. As all studies in the analysis evaluated both tests in all patients this comparison should not be biased by differences between studies.

RESULTS

Results of the search

The results of electronic database and handsearching are outlined in **Figure 1**. There were no disagreements between authors about either the number of studies eligible for inclusion, nor data results. The PubMed searches identified 2899 citations and 78 from CENTRAL. Of these we considered 17 relevant to the purpose of our review and we retrieved 17 full-text articles for more details. We subsequently excluded 13 articles (see the Characteristics of excluded studies table). One additional study was included through the reference lists of eligible studies. Only five studies fulfilled our inclusion criteria. All of this studies compared culture with PCR and only one compared culture with PCR and DFA. The details of all studies included are reported in Characteristics of included studies.

Figure I. Flow of studies identified in literature search



Methodological quality of included studies

All included studies satisfied the QUADAS criteria of including study populations that represented the intended target population (patients suspected of having pneumonia caused by *Legionella* spp) and an acceptable reference standard (culture). In tree studies the delay between tests was not reported but likely to be only short delay (Cloud 2000; Lisby 1994; Pasculle 1989). In two studies there was no delay between tests because the same samples were tested by both index test and reference standard (Edelstein 1987; Hayden 2001). In all included studies, all patients who received the index test were also evaluated by the reference standard. No patients were verified with a second or third reference standard because disease status (LD) was diagnosed only by culture. Differential verification was therefore also avoided in all studies. Incorporation bias; which occurs when the index test is incorporated in a composite reference standard, often leading to overestimation of diagnostic test accuracy, was not present in any study. It was not reported if culture results were known at the time of index test in four studies (Cloud 2000; Hayden 2001; Lisby 1994; Pasculle 1989). It was not reported if index test results were known at the time of culture in tree studies (Cloud 2000; Hayden 2001; Pasculle 1989) In all included studies the data available during the study of diagnostic test accuracy was the same as that which would have been available in normal clinical practice. There were no incomprehensible results in any included study. There were no withdrawals from the studies.

Figure 2. Methodological quality of the five included studies.

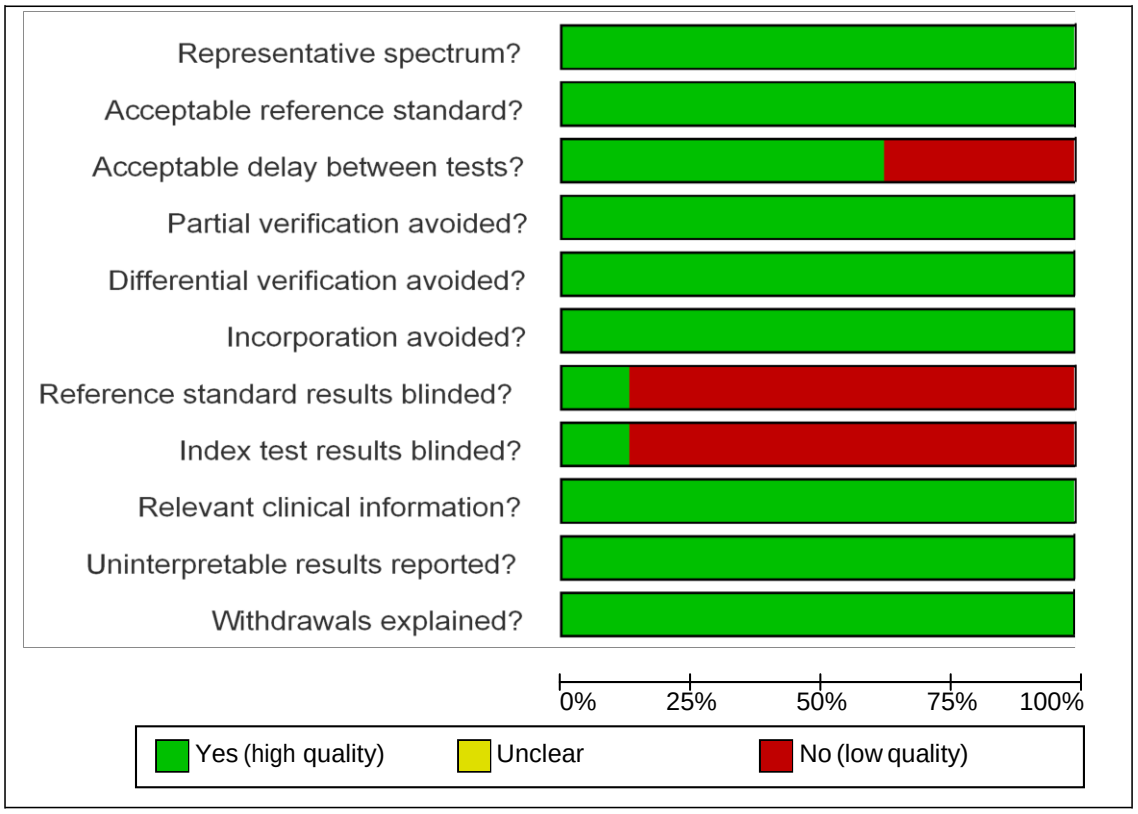
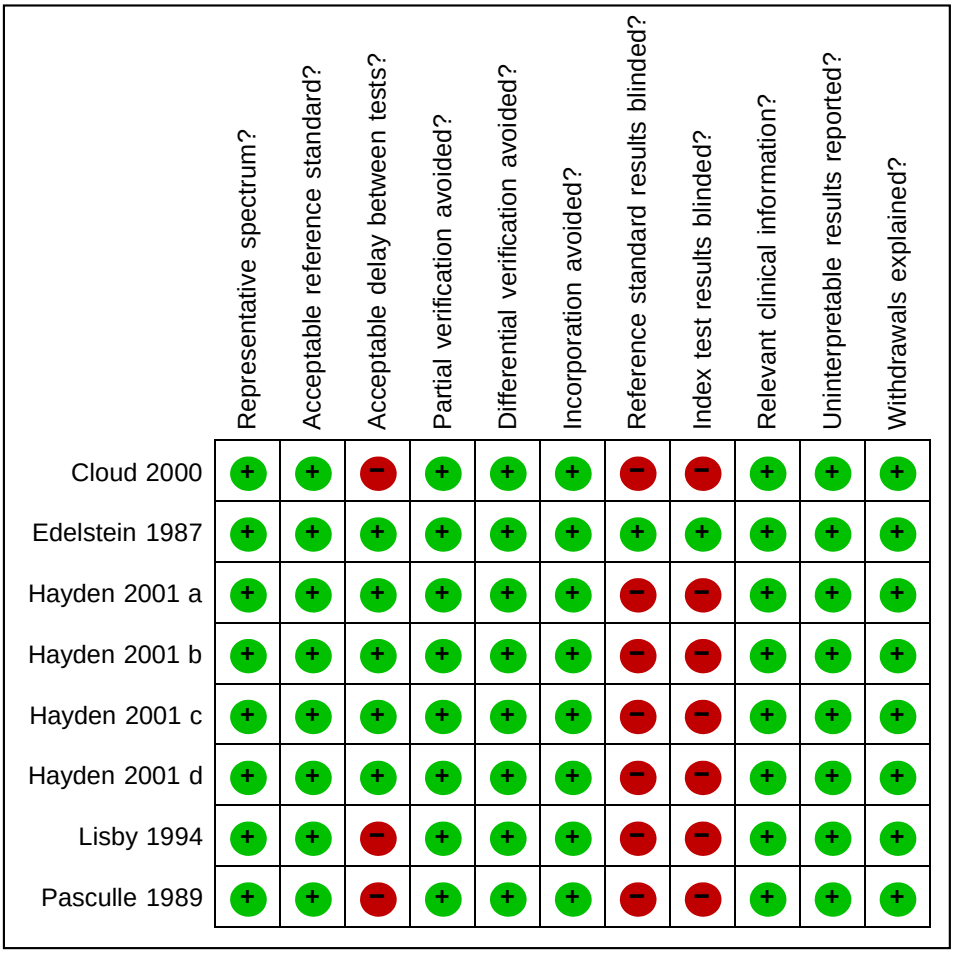


Figure 3. Methodological quality summary of studies: review authors' judgments about each methodological quality item for each included study using the Quality Assessment of Diagnostic Accuracy Studies tool



Findings

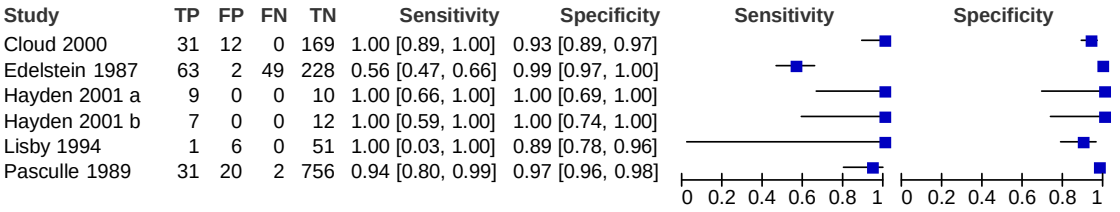
We identified 5 studies that evaluated PCR e DFA tests, a total of 1457 clinical samples from patients suspected of having pneumonia caused by *Legionella* spp. All studies were designed to evaluate PCR and DFA tests to detect *Legionella* in clinical specimens, comparing it with culture (reference standard). Forest plot of the study estimates of sensitivity and specificity for PCR and DFA is shown in Figure 4.

Figure 5 depicts the SROC plot of sensitivity and specificity, arranged by test comparison, for all studies identified and included in the meta-analysis.

Factors such as patient characteristics, study design, test application (criteria for positive test) and study quality factors demonstrate no heterogeneity between studies included in this review.

Figure 4. Forest plots PCR results for LD.
The squares represent the sensitivity and specificity of one study, the black line its confidence interval. FN: false negative; FP: false positive; TN: true negative; TP: true positive

PCR



DFA

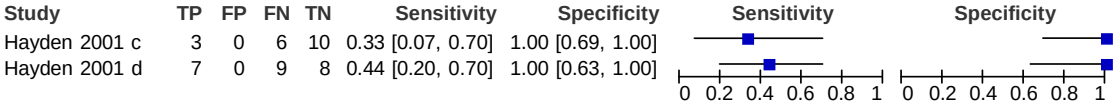
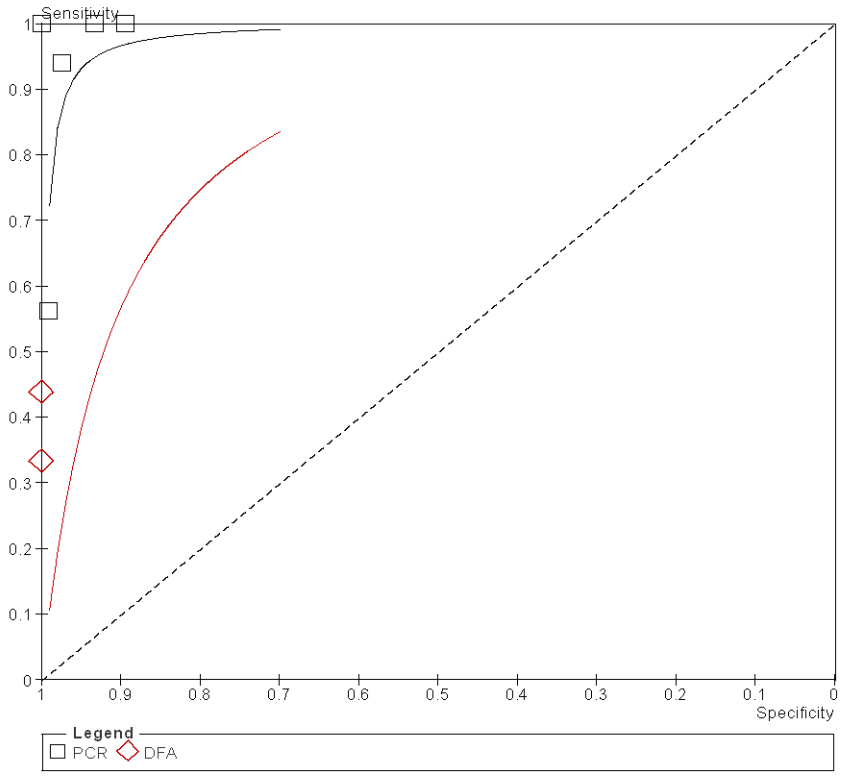


Figure 5. Summary receiver operator curve plot of sensitivity versus specificity for performance of PCR and DFA tests. Each symbol represents a study, with the height and width of each symbol being proportional to the inverse standard error of the sensitivity and specificity respectively.



Protein Chain Reaction (PCR)

PCR was compared with culture in 5 studies (1457 clinical samples) (Cloud 2000; Edelstein 1987; Hayden 2001; Lisby 1994; Pasculle 1989). Figure 4 and 5, shows the Forest plots and SROC plots of the sensitivity and specificity estimates for PCR in the studies mentioned above. The sensitivity of PCR varied from 56% to 100% and the specificity from 89% to 100%. The pooled sensitivity was 74% (95% IC 67%-80%), and the specificity 97% (95%IC 96%-80%).

Direct fluorescent antibody (DFA) staining

DFA was compared with gold standard culture method in 1 study (43 clinical samples) (Hayden 2001). Figure 4 and 5, shows the Forest plots and SROC plots of the sensitivity and specificity estimates for DFA in the studies mentioned above. The DFA assay while requiring a similar time frame for testing was shown to have a sensitivity varied from 33% to 44% and the specificity from 100%. Pooled sensitivity was 40% (95% IC 21%-61%) and the specificity 100% (95% IC 81%-100%).

Summary of results

Summary of results: Accuracy of diagnostic tests for legionnaires´ disease

Review question: Any of the listed index tests where they were compared with the reference standard of Legionnaires´ disease

Patient population:

Patients suspected of suffering from LD or patients with laboratory confirmed LD

Geographical location: Investigations performed in hospital or in Laboratory

Index test :

- Direct fluorescent antibody (DFA) staining;
- Urinary antigen detection (immunochromatography);
- Serological testing;
- Protein Chain Reaction (PCR)

Reference standard: Culture

Included studies: PCR (5 studies, 1457 clinical samples); DFA (1 study, 43 clinical samples)

Limitations

- Limited number of included studies (5 studies); some studies had small samples size**;** only PCR and DFA were evaluated in detail. No studies were found investigating urinary antigen detection (immunochromatography) and serological testing as all studies in the analysis evaluated both tests in all patients this comparison should not be biased by differences between the studies.

Results

PCR		**DFA**		**Summary effect (95% CI)**	
TP	142	**TP**	10	**PCR Sensitivity**	0.74 (0.67 to 0.8)
FP	40	**FP**	0	**PCR Specificity**	0.97 (0.96-0.98)
FN	51	**FN**	15	**DFA Sensitivity**	0.4 (0.21-0.61)
TN	1266	**TN**	18	**DFA Specificity**	1.0 (0.81-1.0)
Total	1499	***Total***	43		

Conclusions and comments

PCR is an attractive test, sensitive specific and convenient, we need further studies to approach the place this PCR test in the diagnosis of multifaceted atypical pneumonia. The availability of real-time PCR offers the potential for dramatically increasing the speed with which Legionellosis can be diagnosed.

The DFA assay, while was shown to have a relatively low sensitivity (40%), allows for a probable diagnosis.

Applicability of tests in clinical practice
We reviewed the diagnostic accuracy of PCR comparing it to culture, the reference method for Legionnaires ´disease. There is evidence that PCR is a sensitive and specific test, valuable for the detection of cases of Legionelosis and it is likely that in the near future PCR is a test of choice in all laboratories.
Costs
None of the studies included a cost-effectiveness evaluation. PCR is known to be more expensive than Culture.

Note: CI: confidence interval; DFA: direct fluorescent antibody; FN: false negative; FP: false positive; PCR: protein chain reaction; TN: true negative; TP: true positive

DISCUSSION

The diagnostic methods have been improved since *Legionella pneumophila* was first described; there are high sensitivity and specificity tests that allow a rapid and effective diagnosis in laboratorial routine. An early diagnosis of LD is crucial for a therapeutic decision since many first-line antibiotics commonly used for bacterial pneumonias management (i.e., beta-lactams) are ineffective against *Legionella* species. We conducted a systematic review of the accuracy of different diagnostic tests for Legionnaires' disease compared to the gold standard, culture method. This systematic review found a relative scarcity of studies that met the inclusion criteria and only five studies fulfilled our inclusion criteria. All studies compared PCR, and only one compared PCR and DFA to culture. These primary studies provide evidence to evaluate the performance of the tests for LD.

Cloud (Cloud, 2000), evaluated 186 respiratory samples from patients suspected of having pneumonia caused by *Legionella* spp., 31 sputum specimens, 66 bronchial washing specimens, 74 bronchoalveolar lavage (BAL) specimens, 8 pleural fluid specimens, and 7 lung tissue specimens. 26 respiratory samples (13 BAL, 7 sputum, 4 bronchial washing, and 2 pleural fluid specimens) were spiked in order to contain a final concentration of approximately 102 to 103 organisms of *L. pneumophila* (ATCC 33512) per ml. A PCR assay was developed, in which the target for the test is the 16S rRNA gene, which exists in multiple copies per genome, thus improving the sensitivity of detection. Several medically relevant *Legionella* species including *L. pneumophila*, *L. micdadei*, *L. bozemanii*, *L. longbeachae*, *L. feeleii*, and *L. dumoffii* can be detected without the use of culture.

Edelstein (Edelstein, 1987), studied a total of 112 clinical specimens (positive samples previously selected) collected from 64 patients that were used as positive samples. At the same time, a total of 230 negative specimens were selected; Negative samples were confirmed by culture and DFA techniques. These samples were selected from patients with clinical suspicion of LD and were cryopreserved at -70°C for 2 to 8 years without specific preservative. All 342 clinical samples were randomly included in the study by computer-generated random-number list. True identity of the samples was unknown. Gen Probe developed hybridization probes directly on clinical specimens - Gen-Probe Rapid Diagnostic System for *Legionella* spp. This kit contains cDNA labeled with ^{125}I, which specifically hybridizes to the rRNAs of all *Legionella* species.

Pasculle (Pasculle 1989) studied a total of 809 clinical specimens, mostly sputum samples, which were submitted to their laboratory between 2 April and 31 October 1987 for *Legionella* testing. They performed culture, DFA, and DNA probe testing (Gen-Probe kit used by Edelstein in the previously mentioned study).

Lisby (Lisby 1994) studied routine clinical bronchial fluid samples from 51 patients (30 male and 21 female, median age 61 years) with clinical suspicion of Legionnaire's disease diagnosis were performed by culture technique. Thirty-seven children (median age 1 year) with a suspected viral pulmonary infection were included as negative controls. A polymerase chain reaction (PCR) assay for the detection of *Legionella* spp in clinical bronchial fluid samples was developed DNA from patients and controls were analyzed blindly. The PCR was able to detect a 375 bp fragment of the 16S RNA gene, equivalent to 10 cfu in simulated clinical bronchial fluid and blood samples.

Hayden (Hayden, 2001) studied a total of 43 archived specimens from 35 patients including 19 bronchoalveolar lavage (BAL) specimens, and 24 formalin-fixed, lung biopsy specimens. BAL

specimens were tested by LightCycler PCR (LC-PCR) methods, and by a direct fluorescent antibody (DFA) assay, which detects *L. pneumophila* serogroups 1 to 6 and several other *Legionella* species. Tissue sections were tested by the two LC-PCR methods, by DFA, by an in situ hybridization (ISH) assay, specifically designed to detect *L. pneumophila*, and by Warthin-Starry (WS) staining. The results were compared to the "gold standard" method of bacterial culture. The samples were frozen and randomly selected. *Legionella* species detection, directly from clinical specimens, were performed by real-time PCR (LightCycler (Roche Molecular Biochemicals, Indianapolis, Ind.), using a primer-probe sets tested against a total of 17 different known strains. The target was a *Legionella* genus (5S rDNA) of all species, with all *L. pneumophila* serotypes detected by the L. pneumophila species-specific (mip) primers and probes. Optimized PCR conditions, as well as dilution studies to evaluate sensitivity and plasmid construction, were performed using using *L. pneumophila* serogroup 1 (ATCC 33152). Other strains of *Legionella*, used for validation of the assay included *L. pneumophila* serogroups 1 to 6, as well as several other strains of *Legionella*, representing the most commonly isolated non-*L. pneumophila* species. With conventional culture serving as the "gold standard," the results of LC-PCR were compared to direct fluorescent antibody (DFA) assay for the detection of *Legionella* species in BAL specimens, and open lung biopsy specimens. According to QUADAS items, these studies were classified as having high quality methodology. When the reference standard was interpreted knowing the index test results, this may have led to the overestimation of diagnostic test accuracy the studies.

Our review showed that PCR have high levels of sensitivity and specificity to detect all species of *Legionella*. Our key findings are presented in Summary of results. The sensitivity of PCR varied from 56% to 100% and the specificity from 89% to 100%. The pooled sensitivity was 74% (95% IC 67%-80%), and the specificity 97% (95%IC 96%-80%). The sensitivity of DFA varied from 33% to 44% and specificity was 100%. The pooled sensitivity was 40% (95% IC 21%-61%) and the specificity 100% (95% IC 81%-100%).

The gold standard technique has low sensitivity, of approximately 50 to 60% related to the fastidious nature of the bacteria, which requires 3-5 days to form visible colonies and must be examined by an experienced professional. There are even species that do not grow in the culture medium. The fact that the majority of patients have already started antibiotic therapy prior to sample collection is also associated with the low sensitivity of the gold standard. All these problems cause a delayed laboratory response, not responding in time to the demands of the diagnosis of a serious condition. Although the above characteristics, culture have 100% specificity for LD diagnosis.

In conclusion, we identified only a limited number of studies that directly compared PCR and DFA versus culture for the early detection of LD. The overall methodological quality of these studies was high to moderate. Due to the small number of included studies, we could not reach a definitive conclusion. However, according to the results obtained in this review, DFA was shown to have a relatively low sensitivity. PCR is an attractive test, easy to perform, providing high sensitivity and high specificity results in only a few hours.

AUTHORS' CONCLUSIONS

Implications for practice

PCR has a high sensitivity and specificity, allowing the diagnosis of infections caused by *Legionella* spp. This review seems to demonstrate that in near future, PCR will be the test that meets the requirements of quickly and efficiently needed for a rapid diagnosis of *Legionella* infections. However standardization is required for biological samples. Culture is always required for epidemiological studies, strains molecular typing and allows antimicrobial susceptibility testing whenever required.

Implications for research

Additional well designed studies are needed in order to achieve the best standard test that enables optimization of *Legionella* infection diagnostic. The results of such experiments would be very helpful to clinicians and microbiologists, which are currently faced with different tests and might help to establish standard methods that can be used not only in research but in daily routine practice.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies (*ordered by study ID*)

Cloud 2000

Clinical features and settings	**Clinical features** Patients suspected of having pneumonia caused by *Legionella* spp. **Settings** Associated Regional and University Pathologists Diagnostic Infectious Diseases Laboratory, Salt Lake City, Utah
Participants	212 respiratory samples
Study design	Observational
Index test	PCR
Reference standard	Culture
Outcomes	True positive, True negative, False positive and False negative results, Sensitivity, Specificity.
Notes	

Table of Methodological Quality

Item	Authors´ judgement	Description
Representative spectrum? All tests	Yes	Patients suspected of having pneumonia caused by *Legionella* spp.
Acceptable reference standard? All tests	Yes	Culture, the gold standard for LD.
Acceptable delay between tests? All tests	No	Not reported.
Partial verification avoided? All tests	Yes	All patients were evaluated by culture.
Differential verification avoided? All tests	Yes	This was not an issue in this study. Disease status (LD) was diagnosed only through culture.
Incorporation avoided? All tests	Yes	This was not an issue in this study. Disease status (LD) was diagnosed only through culture.
Reference standard results blinded? All tests	No	Not reported.
Index test results blinded? All tests	No	Not reported.
Relevant clinical information? All tests	Yes	Relevant clinical information was provided regarding the performance and analysis of both the index and reference tests.
Uninterpretable results reported? All tests	Yes	No results were reported to be uninterpretable.
Withdrawals explained? All tests	Yes	No missing patients.

Edelstein 1987

Clinical features and settings	**Clinical features** Patients suspected of having Legionnaires disease. **Settings** Not reported but likely to be at Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia
Participants	342 clinical samples
Study design	Observational
Index test	PCR
Reference standard	Culture
Outcomes	True positive, True negative, False positive and False negative results, Sensitivity, Specificity.
Notes	

Table of Methodological Quality

Item	Authors´judgement	Description
Representative spectrum? All tests	Yes	Patients suspected of having pneumonia caused by *Legionella* spp.
Acceptable reference standard? All tests	Yes	Culture, the gold standard for LD
Acceptable delay between tests? All tests	Yes	The same samples were tested by both index test and reference standard
Partial verification avoided? All tests	Yes	All patients were evaluated by culture
Differential verification avoided? All tests	Yes	This was not an issue in this study. Disease status (LD) was diagnosed only through culture
Incorporation avoided? All tests	Yes	This was not an issue in this study. Disease status (LD) was diagnosed only through culture
Reference standard results blinded? All tests	Yes	Technicians performing the assay had no knowledge of the true identity of the samples
Index test results blinded? All tests	Yes	Technicians performing the assay had no knowledge of the true identity of the samples
Relevant clinical information? All tests	Yes	Relevant clinical information was provided regarding the performance and analysis of both the index and reference tests
Uninterpretable results reported? All tests	Yes	No results were reported to be uninterpretable.
Withdrawals explained? All tests	Yes	No missing patients.

Hayden 2001

Clinical features and settings	**Clinical features** Positive *Legionella* culture specimens and culture- negative for *Legionella* **Setting** Mayo Clinic, Rochester, Minnesota	
Participants	A total of 43 archived specimens from 35 patients were evaluated	
Study design	**Observational**	
Index test	PCR and Direct fluorescent antibody (DFA) staining;	
Reference standard	Culture	
Outcomes	True positive, True negative, False positive and False negative results, Sensitivity, Specificity	
Item	**Authors´judgement**	**Description**
Representative spectrum? All tests	Yes	Patients suspected of having pneumonia caused by *Legionella* spp.
Acceptable reference standard? All tests	Yes	Culture, the gold standard for LD
Acceptable delay between tests? All tests	Yes	The same samples were tested by both index test and reference standard
Partial verification avoided? All tests	Yes	All patients were evaluated by culture
Differential verification avoided? All tests	Yes	This was not an issue in this study. Disease status (LD) was diagnosed only through culture
Incorporation avoided? All tests	Yes	This was not an issue in this study. Disease status (LD) was diagnosed only through culture
Reference standard results blinded? All tests	No	Not reported
Index test results blinded? All tests	No	Not reported
Relevant clinical information? All tests	Yes	Relevant clinical information was provided regarding the performance and analysis of both the index and reference tests
Uninterpretable results reported? All tests	Yes	No results were reported to be uninterpretable.
Withdrawals explained? All tests	Yes	No missing patients.

Lisby 1994

Clinical features and settings	**Clinical features** Patients with clinical findings suggestive of Legionnaire's disease **Settings** Department of Clinical Microbiology, Herlev Hospital, Denmark
Participants	88 Bronchial fluid samples from 51 patients with clinical findings suggestive of Legionnaire's disease
Study design	Observational
Index test	PCR
Reference standard	Culture
Outcomes	True positive, True negative, False positive and False negative results, Sensitivity, Specificity.
Notes	

Table of Methodological Quality

Item	Authors´ judgement	Description
Representative spectrum? All tests	Yes	Patients suspected of having pneumonia caused by *Legionella* spp.
Acceptable reference standard? All tests	Yes	Culture, the gold standard for LD.
Acceptable delay between tests? All tests	No	Not reported..
Partial verification avoided? All tests	Yes	All patients were evaluated by culture.
Differential verification avoided? All tests	Yes	This was not an issue in this study. Disease status (LD) was diagnosed only through culture.
Incorporation avoided? All tests	Yes	This was not an issue in this study.Disease status (LD) was diagnosed only through culture.
Reference standard results blinded? All tests	No	Not reported.
Index test results blinded? All tests	Yes	Not reported.
Relevant clinical information? All tests	Yes	Relevant clinical information was provided regarding the performance and analysis of both the index and reference tests.
Uninterpretable results reported? All tests	Yes	No results were reported to be uninterpretable.
Withdrawals explained? All tests	Yes	No missing patients.

Pasculle 1989

Clinical features and settings	**Clinical features** Patients suspected of having Legionnaires disease. **Settings** Clinical Microbiology Laboratory, Presbyterian-University Hospital, Pittsburgh, Pennsylvania
Participants	809 clinical specimens
Study design	Observational
Index test	PCR
Reference standard	Culture
Outcomes	True positive, True negative, False positive and False negative results, Sensitivity, Specificity.
Notes	

Table of Methodological Quality

Item	Authors´judgement	Description
Representative spectrum? All tests	Yes	Patients suspected of having pneumonia caused by *Legionella* spp.
Acceptable reference standard? All tests	Yes	Culture, the gold standard for LD
Acceptable delay between tests? All tests	No	Not reported.
Partial verification avoided? All tests	Yes	All patients were evaluated by culture
Differential verification avoided? All tests	Yes	This was not an issue in this study. Disease status (LD) was diagnosed only through culture
Incorporation avoided? All tests	Yes	This was not an issue in this study. Disease status (LD) was diagnosed only through culture
Reference standard results blinded? All tests	No	Not reported.
Index test results blinded? All tests	No	Not reported.
Relevant clinical information? All tests	Yes	Relevant clinical information was provided regarding the performance and analysis of both the index and reference tests
Uninterpretable results reported? All tests	Yes	No results were reported to be uninterpretable.
Withdrawals explained? All tests	Yes	No missing patients.

Characteristics of excluded studies

Study	Reason for exclusion
Elveradl 2013	The reference Standard includes not only the culture
Finkelstein 1993	The reference Standard includes not only the culture; No adequate outcome
Morozumi 2006	No adequate outcome
Diederen 2008	The reference Standard includes not only the culture
Laussucq 1988	Not a diagnostic accuracy design
Lindsay 2004	The reference Standard includes not only the culture
Kasandjian 1997	Reference Standard not apply to all patients
Bangsborg 1990	Not a comparison with reference standard; No adequate outcome
Chiba 1998	No adequate outcome
Formica 2001	Not a comparison with reference standard; No adequate outcome
Van Der Eerden 2005	Not a comparison with reference standard; No adequate outcome
Kawanami 2011	Isolated case
Sard 2012	No adequate outcomes

ADDICTIONAL TABLES

QUADAS methodological items and operational definitions

Methodological variable	Operational definition/information required from each study
1. Representative spectrum (*spectrum bias*)	When included patients did not represent the intended targeted population, this may have led to an under- or overestimation of diagnostic accuracy depending on the difference between the targeted and included populations. The target spectrum in our review was patients suspected of suffering from LD or patients with laboratory confirmed LD. This was scored 'yes' if study participants included only patients suspected of suffering from LD or patients with laboratory confirmed LD
2. Acceptable reference standard	An imperfect reference standard may have resulted in misclassification of disease positives and disease negatives. For the purpose of this review, studies had an acceptable reference standard if they used culture as the reference standard
3. Acceptable delay between tests (*disease progression bias*)	Disease may have progressed to a more advanced stage if a significant time interval between index and reference tests was observed, thereby leading to disease progression bias. This was scored as 'yes' if the delay between test was short (i.e. less than three months)
4. Partial verification avoided (*verification bias*)	Partial verification bias usually leads to an overestimation of sensitivity, although its effect on specificity varies. This item was scored 'yes' if all patients who received the index test were also evaluated by the reference standard
5. Differential verification avoided	This was scored 'yes' if no patients were verified with a second or third reference standard
6. Incorporation avoided (*incorporation bias*)	This bias usually leads to an overestimation of diagnostic test accuracy. Incorporation bias was deemed to have existed if the index test was incorporated in a composite reference standard. Studies were scored 'yes' if their classification of disease status did not directly involve the results of the index test
7. Reference standard results blinded (*information bias*)	When the reference standard was interpreted knowing the index test results, this may have led to the overestimation of diagnostic test accuracy. Studies were scored 'yes' if blinding of the reference standard was explicitly stated in the article or if this was acknowledged by authors in subsequent personal communication. Otherwise, the studies were marked 'unclear', unless blinding was explicitly stated to be absent
8. Index test results blinded (*information bias*)	When the index test results were interpreted without the

	knowledge of results of the reference standard, or with more information than in practice, this may have resulted in bias, usually leading to an overestimation of diagnostic accuracy. This item was scored 'yes' if blinding of the index test was explicitly stated in the article or if this was acknowledged by authors in subsequent personal communication. Otherwise, the studies were marked 'unclear', unless blinding was explicitly stated to be absent
9. Relevant clinical information (*information bias*)	The availability of clinical data during interpretation of test results may have affected estimates of test performance. This item was scored 'yes' if the data available during the study of diagnostic test accuracy was the same as that which would have been available in normal clinical practice
10. Uninterpretable results explained	This item was scored 'yes' if uninterpretable results were explained or if there were no uninterpretable results present. This item was scored 'no' if uninterpretable results were found but not explained
11. Withdrawals explained	Excluding patients from the study may have led to an overestimation of diagnostic accuracy. This item was scored 'yes' if withdrawals were explained or if there were no withdrawals from the study. This item was scored 'no' if there were withdrawals from the study, but these were unexplained

A P P E N D I C E S

Appendix 1

Search strategy for the Cocrhrane Central Register of Controlled Trials

- "Legionella","Legionellosis",
- "Legionnaires'disease",
- "Legionella AND diagnosis",
- "Legionellosis AND diagnosis",
- "Legionnaires'disease AND Diagnosis"

Appendice 2

Search strategy for the MEDLINE (PubMed)

- #1 (legionnaires´disease OR legionellosis OR legionella) AND microbiological-diagnosis
- #2 (legionnaires´disease OR legionellosis OR legionella) AND microbiological-diagnosis AND study
- #3 (legionnaires´disease OR legionellosis OR legionella) AND healthcare-associated AND diagnosis
- #4 (legionnaires´disease OR legionellosis OR legionella) AND microbiological-methods
- #5 (legionnaires´disease OR legionellosis OR legionella) AND culture AND study
- #6 (legionnaires´disease OR legionellosis OR legionella) AND urinary-antigen